

REMARKS

In response to the Office Communication dated May 9, 2001 and in accordance with the provisions in 37 C.F.R. §1.821, Applicants submit herewith a substitute paper and an initial computer readable copy of the Sequence Listing, along with a Statement Under 37 C.F.R. §1.821(f), stating that these copies are identical. A copy of the Notice to Comply is also enclosed.

Applicants respectfully direct the Examiner's attention to the changes made to the initial Sequence Listing filed with the application. In the first page of the Sequence Listing, Sections <120>, <130>, <140>, <141> and <160> have been amended to insert the correct title, reference number, application serial number, international filing date and the number of sequences.

In SEQ ID NO: 3, Section <213> has been amended to read "synthetic." Support for this amendment can be found throughout the specification and the claims, e.g., claim 5.

In SEQ ID NO: 10, Section <211> has been amended to insert the correct number of residues in accordance with the sequence of SEQ ID NO: 10; Section <223> has been added and the sequence has been modified in order to comply with the requirements for modified residues.

In SEQ ID NO: 11, repeated Sections <210>, <211>, <212> and <213> have been deleted to correct an apparent typographical error.

In SEQ ID NO: 14, Sections <210>, <211>, <212>, <213> have been amended to insert the correct information regarding the characteristics of this sequence. Support for this amendment is found at page 13 lines 13-18 of the specification.

In SEQ ID NOS: 15-31, Section <210> of these sequences has been amended to insert the correct number of residues of these sequences.

In SEQ ID NO: 22, Section <223> has been added and the sequence has been modified in order to comply with the requirements for modified residues.

In SEQ ID NO: 26, Section <211> has been amended to insert the correct number of residues. Section <223> has been added and the sequence has been modified in order to comply with the requirements for modified residues. Support for these amendments can be found at page 14, lines 2 and 15 of the specification.

In SEQ ID NOS: 27, 28, 29, 30, 31, 32, 35, 36, 37 and 38, Section <223> has been added and the sequences have been modified in order to comply with the requirements for modified residues.

In SEQ ID NO: 32, Section <211> has been amended to insert the correct number of residues.

SEQ ID NOS: 39-44 are added. These sequences are disclosed in the specification at page 12, line 17, page 30, line 24, page 31, lines 9 to 10 and page 33, lines 2 to 3, respectively.

Applicants respectfully submit that the content of the substitute paper copy and the initial computer copy of the sequence listing does not introduce new matter.

In connection with the submitted Sequence Listing, Applicants have also amended the specification and the claims.

Specifically, the sequence identifiers for SEQ ID NOS: 39-44 have been inserted at page 12, line 17, page 30, line 24, page 31, lines 9 to 10 and page 33, lines 2 to 3, respectively.

The hydroxyl group of SEQ ID NO. 24, disclosed at page 13, line 31 and claim 5, has been deleted. As described at page 14, line 13, compound (14) has a free carboxyl at the C-

terminal. As also indicated at page 9 line 18, the peptides of the present invention are typically amidated at the C-terminal.

The acetyl group at the N-terminus of compound (16) at page 14 line 2 has been deleted. Support for such amendment is found at page 14 line 15 of the specification. A similar amendment has been made to claim 5.

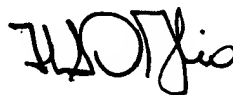
Page 14, line 17 of the specification has been amended to clarify (O) in the sequence of compound 22. This amendment is supported in the specification at page 14, line 8.

Certain typographical errors in the sentence at page 14 lines 19 and 20 have been corrected. Support for these corrections can be found at page 13, line 27 and page 14 lines 3-7.

It is respectfully submitted that the foregoing amendments do not introduce new matter. Attached hereto is a marked-up version of the changes made to the specification and claims by the instant amendment. The attached page is captioned "**Version with Markings to Show Changes Made.**"

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



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Enclosures:

Exhibit A (clean copy of the amended pages of the specification)

Exhibit B (marked-up version of the amended pages of the specification)

Serial No: 09/673,490
Date: July 9, 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

Pages 12, 13, 14, 30, 31 and 33 of the specification have been amended as indicated in Exhibit B, captioned as "Marked-Up Version of the Amended Pages of the Specification."

In the claims:

Claim 5 has been amended as follows:

5. An isolated, synthetic or recombinant ω -conotoxin peptide according to claim 1 having the following sequence:

CKSKGAKCSKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 5]
CKSKGAKCSRLMYDCCSGSCSGTVGRC	[SEQ ID NO: 6]
CKSKGAKCDRLMYDCCSGSCSGTVGRC	[SEQ ID NO: 7]
CRSKGAKCSKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 14]
CKSKGARCSKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 15]
CKSKGAQCSKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 16]
CKSKGAKCSKLMYDCCSGSCSGAVGRC	[SEQ ID NO: 17]
CKSKGAKCDKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 18]
CKYKGAKCSRLMYDCCSGSCSGTVGRC	[SEQ ID NO: 19]
CKSKGAKCSKLAYDCCSGSCSGTVGRC	[SEQ ID NO: 20]
CKSKGAKCSKLMYDCCTGSCSGTVGRC	[SEQ ID NO: 21]
CKSKDaIAKCSKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 22]
CKSKGAKCSKLMYDCCSGSCSGTVGRCY	[SEQ ID NO: 23]
CKSKGAKCSKLMYDCCSGSCSGTVGRC[-OH]	[SEQ ID NO: 24]
YCKSKGAKCSKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 25]
[Ac-]CKSKGAKCSKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 26]
CKSKGAKCSKLNleYDCCSGSCSGTVGRC	[SEQ ID NO: 27]

CKSKGAKCSRLNleYDCCSGSCSGTVGRC [SEQ ID NO: 28]
CKYKGAKCSRLNleYDCCSGSCSGTVGRC [SEQ ID NO: 29]
CKSKGAKCSKLOmhserYDCCSGSCSGTVGRC [SEQ ID NO: 30]
CKSKGAKCSKLOmserYDCCSGSCSGTVGRC [SEQ ID NO: 31]
CKSKGAKCSKLM(O)YDCCSGSCSGTVGRC [SEQ ID NO: 32]



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IN THE SPECIFICATION:

In view of this novel confirmation of loop 4 and the stabilisation between loops 2 and 4 of CVID, one preferred group of derivatives are those which maintain an orientation of loop 4 similar to that seen in CVID. A further preferred group of derivatives are those ω -conotoxins which have an interaction or interactions between loops 2 and 4 which stabilise the confirmation of loops 2 and 4. Those skilled in the art may readily determine the three-dimensional structure of particular peptides, the orientation of loop 4 and interactions between the loops.

Another preferred group of derivatives are those which maintain or only have conservative substitutions at residues 10, 11, 22 and 23 of CVID.

In another embodiment of the present invention there is provided a chimeric ω -conotoxin peptide in which one or more of loops 1 to 3 of conotoxin CVID have been substituted with the corresponding loop of a different ω -conotoxin.

15

A preferred group of derivatives of CVID are those ω -conotoxin peptides which maintain certain residues of CVID. These derivatives are represented in the following sequence

CxxxGxxCxKLxYxCCxSCSGxVGRC [SEQ ID NO: 39]

where each x may be any other amino acid and up to one x may be a deletion. Preferred selections for x would be the corresponding natural amino acids from ω -cono-peptides with N-type BSCC selectivity and conservative substitutions or alanine substitutions of those amino acids, all of which may also have modified side chains. For example, methionine may be replaced with O-methyl serine or O-methyl homoserine.

25 Some known conotoxins are as follows:

MVIIA (SNX-III)

CKGKGAKCSRLMYDCCTGSCRSKGC [SEQ ID NO: 8]

MVIIC

CKGKGAPCRKTMVDCCSGSCGRRGKC [SEQ ID NO: 9]

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GVIA CKSOGSSCSOTSYNCCRSCNOYTKRCY [SEQ ID NO: 10]

In the sequence for GVIA the "O" refers to 4-hydroxy proline (Hyp). This amino acid residue results from post translational modification of the encoded peptide and is not directly encoded by the nucleotide sequence.

Chimeric ω -conotoxins contemplated by the present invention include DADD, DAGD and GGGD. Where a D, A or a G represent loops selected from CVID, MVIIA or GVIA respectively. Accordingly DADD corresponds to loops 1, 3 and 4 being selected from CVID and loop 2 being selected from MVIIA, this chimeric ω -conotoxin is the same as R¹⁰-CVID.

A number of other ω -conotoxin peptides according to the invention were found to be encoded by mRNA isolated from *Conus catus* according to the general procedure described in Example 3. These encoded peptides were synthesised by standard procedures and may be considered as derivatives of CVID, the sequences are as follows:

(4) CRSKGAKCSKLMYDCCSGSCSGTVGRC [SEQ ID NO: 14]
(5) CKSKGARCSKLMYDCCSGSCSGTVGRC [SEQ ID NO: 15]
20 (6) CKSKGAQCSKLMYDCCSGSCSGTVGRC [SEQ ID NO: 16]
(7) CKSKGAKCSKLMYDCCSGSCSGAVGRC [SEQ ID NO: 17]

Examples of other derivatives of CVID include the following sequences:

25	(8)	CKSKGAKCDKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 18]
	(9)	CKYKGA KC SRLMYDCCSGSCSGTVGRC	[SEQ ID NO: 19]
	(10)	CKSKGAKCSKLAYDCCSGSCSGTVGRC	[SEQ ID NO: 20]
	(11)	CKSKGAKCSKLMYDCCTGSCSGTVGRC	[SEQ ID NO: 21]
	(12)	CKSKDaIAKCSKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 22]
30	(13)	CKSKGAKCSKLMYDCCSGSCSGTVGR CY	[SEQ ID NO: 23]
	(14)	CKSKGAKCSKLMYDCCSGSCSGTVGRC	[SEQ ID NO: 24]

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- (15) YCKSKGAKCSKLMYDCCSGSCSGTVGRC [SEQ ID NO: 25]
 (16) CKSKGAKCSKLMYDCCSGSCSGTVGRC [SEQ ID NO: 26]
 (17) CKSKGAKCSKLNleYDCCSGSCSGTVGRC [SEQ ID NO: 27]
 (18) CKSKGAKCSRLNleYDCCSGSCSGTVGRC [SEQ ID NO: 28]
 5 (19) CKYKGAKCSRLNleYDCCSGSCSGTVGRC [SEQ ID NO: 29]
 (20) CKSKGAKCSKLOmhserYDCCSGSCSGTVGRC [SEQ ID NO: 30]
 (21) CKSKGAKCSKLOmserYDCCSGSCSGTVGRC [SEQ ID NO: 31]
 (22) CKSKGAKCSKLM(O)YDCCSGSCSGTVGRC [SEQ ID NO: 32]

- 10 Compounds (13) and (15) (SEQ ID NOS: 23 and 25) have an additional amino acid at the C-terminal and N-terminal respectively.

Compound (14) (SEQ ID NO: 24) has a free carboxyl at the C-terminal.

- 15 Compound (16) (SEQ ID NO: 26) is acylated at the N-terminal.

Compound (22) (SEQ ID NO: 32) has the methionine residue at position 12 oxidised to its sulfoxide, as indicated by (O).

- 20 Compounds (10), (17), (18), (19), (20) and (21) (SEQ ID NOS: 20, 27, 25, 29, 30 and 31, respectively) represent methionine replacements at position 12.

A preferred group of ω -conotoxin peptides are CVID, compounds (4), (5), (10), (17), (18), (20), and (21). A particularly preferred ω -conotoxin peptides is CVID.

25

In the accompanying sequence listing the amino acids Xaa are as shown in Table 2.

Table 2

SEQ ID NO:	Xaa
SEQ ID NO: 22	Dal

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Table 3 List of synthesised peptides, optimal yields and Mass values.

PEPTIDE	REDUCED YIELD %	OXIDISED YIELD %	EXPECTED MASS (Mr,Da)	OBSERVED MASS (Mr,Da)
CVID	36	35	2755	2755
R ¹⁰ -CVID	36	40	2784	2784
D ⁹ R ¹⁰ -CVID	33	29	2812	2812

Example 3**Isolation and Characterisation of the CVID Gene Sequence****RNA extraction and cDNA synthesis**

Two specimens of *Conus catus* were collected from Lady Elliot Island on the Queensland Great Barrier Reef. The animals were anaesthetised on ice, and dissected to remove the venom duct in a region from the venom bulb to the proboscis. The ducts were sectioned, placed in a buffer containing guanidinium thiocyanate/N-lauroyl sarcosine, then emulsified with manual grinding. Poly-A tailed mRNA was extracted from the mixtures using the Pharmacia Biotech QuickPrep mRNA purification system.

Strand-1 cDNA was 3' end synthesised from the *C. catus* poly-A mRNA templates using a NotI-d(T)₁₈ bifunctional primer (5'-AACTGGAAGAATTTCGCGCCGCAGGAAT₍₁₈₎-3') [SEQ ID NO: 40] (Pharmacia Biotech) in conjunction with Superscript II reverse transcriptase (Gibco BRL). The resultant cDNA templates were used to manufacture double stranded cDNA using a RNaseH/DNA polymerase procedure as per the Pharmacia Biotcch cDNA Timesaver

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protocol. Marathon (Clontech) adaptors were then added to the 5' and 3' ends of the ds-cDNA molecules to complete the cDNA construction. A representation of a complete coneshell venom peptide cDNA molecule is shown in figure 1.

PCR derivation of CVID and related cDNA sequences

PCR was carried out on samples containing ds-cDNA from *C.catus*, the CSRD-301A primer (5' - ATCATCAAAATGAACTGACGTC - 3') [SEQ ID NO:41], the ANCHOR primer (5' - AACTGGAAGAATTCGCGGCCGCGCAGGAAT - 3') [SEQ ID NO: 42] and an appropriate *Tag* polymerase (Biotech International) and buffer (25mM Mg, 100uM deoxy-nucleotides, buffered at pH 8.5) in a thermal cycler (Omnigene) at 95°C/2 mins for 1 cycle, 95°C/30 sec - 55°C/60 sec - 72°C/90 sec for 35 cycles, and 72°C/10 mins for 1 cycle. This PCR produced a heterogeneous DNA product of approximately 380 bp to 500bp. Sequence analysis of clones derived from this PCR product have shown it to contain the sequence CVID as well as other related venom peptide sequences.

Cloning and sequencing of CVID

The DNA product produced from the CSRD-301A-ANCHOR driven PCR of *C. catus* cDNA was electrophoresed in low melting point agarose and excised. The DNA was extracted from the agarose on Qiagen columns, rephosphorylated with T4 DNA kinase (Progen), blunt ended with Klenow polymerase (Progen), and ligated with T4 DNA ligase (Progen) into the multiple cloning site of dephosphorylated *Sma*-1 cut pUC-18 plasmid vector DNA (Pharmacia Biotech). The vector DNA was electrotransformed into Bluescript *E.coli* cells, to produce a library of clones representing the PCR product. Aliquots of the library were plated onto LB_{amp} plates, and individual clones selected and propagated overnight in TB_{amp} broth. Plasmid DNA was purified from the culture using the RPM system (BIO-101), and the PCR DNA inserts within the vector sequenced using the pUC-18 forward and reverse

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additional primers in place of the CSRD-301A primer. These primers were designed to be more specific and were OM-2A (5'-ATC AAA ATG AAA CTG ACG TGT GTG GTG-3') [SEQ ID NO: 43] and Cca-6-3B (5'-GCG TTT TGA TCA GCC ACA TCT ACC TA-3') [SEQ ID NO: 44]. These experiments led to identification of sequences for some of the derivatives of CVID.

Example 4

Radioligand binding assays

Preparation of ^{125}I -GVIA and ^{125}I -MVIIC

Peptides were iodinated using IODO-GEN® (Fraker P.J. *et al.*, 1978) (1,3,4,6-tetrachloro-2a,6a-diphenyl-glycyluril) [Ahmad S.N. *et al.*, 1988, Cruz L.J. *et al.*, 1986], 5 μl (5.75 mg/ml, 17.4 mCi/mg) Na ^{125}I (DuPont NEN®, New research products, Boston) and 25 μl of sodium phosphate buffer (50 mM, pH 7.4) were added to an eppendorf tube coated with IODO-GEN® (Pierce, Rockford, USA) and incubated for 5 mins. The reaction mixture was vortexed and transferred to an eppendorf tube containing 10 μl of the peptide of interest. This mixture was then allowed to react for another 5 min prior to purification by HPLC.

Preparative HPLC of ^{125}I -labelled peptides was performed on a Waters 680 gradient controller equipped with two Waters 510 HPLC pumps and a Waters 481 absorbance detector. Peptides were analysed on Vydac reverse phase C-18 analytical column (4.6 x 250 mm) eluted at 1 ml/min with a linear gradient of 0-67% of solvent B over 100 min: solvent A, 1% TFA (trifluoroacetic acid); solvent B, 90% ACN + 0.09% TFA. Separation was monitored at 214 nm and 1 ml fractions were collected. Fractions of interest were detected with a LKB Wallac 1272 automatic Gamma counter.

To confirm the identity of iodinated peptides, mass spectrometry was performed on a PE-SCIEX API III mass spectrometer (PE-Sciex, Thornhill, Ontario, Canada). HPLC fractions from peptides iodinated with non-radioactive K ^{127}I were injected directly into the mass

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IN THE SPECIFICATION:

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In view of this novel confirmation of loop 4 and the stabilisation between loops 2 and 4 of CVID, one preferred group of derivatives are those which maintain an orientation of loop 4 similar to that seen in CVID. A further preferred group of derivatives are those ω -conotoxins which have an interaction or interactions between loops 2 and 4 which stabilise the confirmation of loops 2 and 4. Those skilled in the art may readily determine the three-dimensional structure of particular peptides, the orientation of loop 4 and interactions between the loops.

Another preferred group of derivatives are those which maintain or only have conservative substitutions at residues 10, 11, 22 and 23 of CVID.

In another embodiment of the present invention there is provided a chimeric ω -conotoxin peptide in which one or more of loops 1 to 3 of conotoxin CVID have been substituted with the corresponding loop of a different ω -conotoxin.

A preferred group of derivatives of CVID are those ω -conotoxin peptides which maintain certain residues of CVID. These derivatives are represented in the following sequence

CxxxGxxCxKLxYxCCxSCSGxVGRC [SEQ ID NO 39]

where each x may be any other amino acid and up to one x may be a deletion. Preferred selections for x would be the corresponding natural amino acids from ω -conopeptides with N-type BSCC selectivity and conservative substitutions or alanine substitutions of those amino acids, all of which may also have modified side chains. For example, methionine may be replaced with O-methyl serine or O-methyl homoserine.

Some known conotoxins are as follows:

MVIIA (SNX-III)

CKGKGAKCSRLMYDCCTGSCRSGKC [SEQ ID NO: 8]

MVIIC

CKGKGAPCRKTMVDCCSGSCGRRGKC [SEQ ID NO: 9]

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GVIA

CKSOGSSCSOTSYNCCRSCNOYTKRCY [SEQ ID NO: 10]

In the sequence for GVIA the "O" refers to 4-hydroxy proline (Hyp). This amino acid residue results from post translational modification of the encoded peptide and is not directly
5 encoded by the nucleotide sequence.

Chimeric ω -conotoxins contemplated by the present invention include DADD, DAGD and GGGD. Where a D, A or a G represent loops selected from CVID, MVIIA or GVIA respectively. Accordingly DADD corresponds to loops 1, 3 and 4 being selected from
10 CVID and loop 2 being selected from MVIIA, this chimeric ω -conotoxin is the same as R¹⁰-CVID.

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15 in Example 3. These encoded peptides were synthesised by standard procedures and may be considered as derivatives of CVID, the sequences are as follows:

- | | | |
|--------|-----------------------------|-----------------|
| (4) | CRSKGAKCSKLMYDCCSGSCSGTVGRC | [SEQ ID NO: 14] |
| (5) | CKSKGARCSKLMYDCCSGSCSGTVGRC | [SEQ ID NO: 15] |
| 20 (6) | CKSKGAQCSKLMYDCCSGSCSGTVGRC | [SEQ ID NO: 16] |
| (7) | CKSKGAKCSKLMYDCCSGSCSGAVGRC | [SEQ ID NO: 17] |

Examples of other derivatives of CVID include the following sequences:

- | | | |
|---------|---|-----------------|
| 25 (8) | CKSKGAKCDKLMYDCCSGSCSGTVGRC | [SEQ ID NO: 18] |
| (9) | CKYKGAKCSRLMYDCCSGSCSGTVGRC | [SEQ ID NO: 19] |
| (10) | CKSKGAKCSKLAYDCCSGSCSGTVGRC | [SEQ ID NO: 20] |
| (11) | CKSKGAKCSKLMYDCCTGSCSGTVGRC | [SEQ ID NO: 21] |
| (12) | CKSKDaIAKCSKLMYDCCSGSCSGTVGRC | [SEQ ID NO: 22] |
| 30 (13) | CKSKGAKCSKLMYDCCSGSCSGTVGRCY | [SEQ ID NO: 23] |
| (14) | CKSKGAKCSKLMYDCCSGSCSGTVGRC OH | [SEQ ID NO: 24] |

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- (15) YCKSKGAKCSKLMYDCCSGSCSGTVGRC [SEQ ID NO: 25]
 (16) ~~Y~~CKSKGAKCSKLMYDCCSGSCSGTVGRC [SEQ ID NO: 26]-
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10 Compounds (13) and (15) (SEQ ID NOS: 23 and 25) have an additional amino acid at the C-terminal and N-terminal respectively.

Compound (14) (SEQ ID NO: 24) has a free carboxyl at the C-terminal.

15 Compound (16) (SEQ ID NO: 26) is acylated at the N-terminal.

Compound (22) (SEQ ID NO: 32) has the methionine residue ^{at position 12} oxidised to its sulfoxide, ^{as indicated} by (O).

Compounds ^{(18), (19), (20)} (10), (17), ^{28, 29} (2), and (21) (SEQ ID NOS: 20, 27, 30 and ³¹ 32, respectively) represent
 20 methionine replacements ^{at position 12}

A preferred group of ω -conotoxin peptides are CVID, compounds (4), (5), (10), (17), (18), (20), and (21). A particularly preferred ω -conotoxin peptides is CVID.

25 In the accompanying sequence listing the amino acids Xaa are as shown in Table 2.

Table 2

SEQ ID NO:	Xaa
SEQ ID NO: 22	Dal

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Table 3 List of synthesised peptides, optimal yields and Mass values.

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 25 (Pharmacia Biotech) in conjunction with Superscript II reverse transcriptase (Gibco BRL). The resultant cDNA templates were used to manufacture double stranded cDNA using a RNaseH/DNA polymerase procedure as per the Pharmacia Biotech cDNA Timesaver

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protocol. Marathon (Clontech) adaptors were then added to the 5' and 3' ends of the ds-cDNA molecules to complete the cDNA construction. A representation of a complete coneshell venom peptide cDNA molecule is shown in figure 1.

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additional primers in place of the CSRD-301A primer. These primers were designed to be more specific and were OM-2A (5'-ATC AAA ATG AAA CTG ACG TGT GTG GTG-3') and Cca-6-3B (5'-GCG TTT TGA TCA GCC ACA TCT ACC TA-3'). These experiments led to identification of sequences for some of the derivatives of CVID.

5

Example 4

Radioligand binding assays

Preparation of 125 I-GVIA and 125 I-MVTIC

10

Peptides were iodinated using IODO-GEN® (Fraker P.J. *et al.*, 1978) (1,3,4,6-tetrachloro-2a,6a-diphenyl-glycoluril) [Ahmad S.N. *et al.*, 1988, Cruz L.J. *et al.*, 1986], 5 μ l (5.75 mg/ml, 17.4 mCi/mg) Na 125 I (DuPont NEN®, New research products, Boston) and 25 μ l of sodium phosphate buffer (50 mM, pH 7.4) were added to an eppendorf tube coated with IODO-GEN® (Pierce, Rockford, USA) and incubated for 5 mins. The reaction mixture was vortexed and transferred to an eppendorf tube containing 10 μ l of the peptide of interest. This mixture was then allowed to react for another 5 min prior to purification by HPLC.

Preparative HPLC of 125 I-labelled peptides was performed on a Waters 680 gradient controller equipped with two Waters 510 HPLC pumps and a Waters 481 absorbance detector. Peptides were analysed on Vydac reverse phase C-18 analytical column (4.6 x 250 mm) eluted at 1 ml/min with a linear gradient of 0-67% of solvent B over 100 min: solvent A, 1% TFA (trifluoroacetic acid); solvent B, 90% ACN + 0.09% TFA. Separation was monitored at 214 nm and 1 ml fractions were collected. Fractions of interest were detected with a LKB Wallac 1272 automatic Gamma counter.

To confirm the identity of iodinated peptides, mass spectrometry was performed on a PE-SCIEX API III mass spectrometer (PE-Sciex, Thornhill, Ontario, Canada). HPLC fractions from peptides iodinated with non-radioactive K 127 I were injected directly into the mass